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A COMPARISON, WITH THE STANDARD PLATE METHOD, OF SOME RAPID METHODS FOR BACTERIOLOGIC ANALYSIS OF MILK*

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The bacterial content of a sample of milk is one of the most important of the many factors that determine the past history of the milk and gives some idea as to its future with reference to keeping quality. To control the quality of the milk supply which the city milk dealer receives, it is very desirable that he should know the bacterial content of the milk from each patron. He should also know the bacterial content of the milk as it leaves each piece of machinery in the milk plant. If this information is obtained, the dealer will know the kind of product which he is placing on the market for consumption. Since milk dealers handle large quantities of milk, the determination of the bacterial content is an enormous task. It would be desirable to use some method of analysis which is more rapid, less expensive of time and equipment than the "standard plate method." It was the purpose of this investigation to compare some of the simpler and quicker methods with the standard plate method in order to determine their practical value.

HISTORICAL REVIEW

The first method used to count bacteria in milk was by means of the microscope.[1] The difficulty which was encountered in this method was in the preparation of the material for examination. It was almost impossible to obtain an even distribution of the milk so that the organisms could be easily distinguished and counted. This difficulty rendered accurate counts impossible at first and the results obtained were only approximately correct.

With the introduction of the plate method by Koch about 1880, the microscopic method of estimating bacteria in milk was almost completely abandoned. The plate method has been adopted as the standard method of milk analysis by the American Public Health Association.[2]

Within the last few years several methods have been devised which have not been widely adopted but are promising. These methods have been devel-

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[1] Hueppe, Ferdinand: Die Methoden der Bacterien-Forschung, 1891.

[2] Standard Methods for the Bacteriological Analysis of Milk, 1916.

oped because of certain disadvantages of the standard plate method. Chief among these disadvantages are: (1) the length of time required for incubation, 48 hours being the shortest; (2) large amount of glassware and culture medium required, as well as other expensive equipment, and (3) the paucity of details regarding the kinds of organisms present.

About 1900, attention was again called to the microscopic method of counting bacteria in richly seeded material. Klein,[3] Winslow,[4] and others have worked on this method with good results. Slack,[5] in 1906, introduced a method in which he sought to determine the bacterial content of milk by counting the bacteria in the sediment obtained by centrifugation. Studying a large number of samples, he worked out an arbitrary ratio between the number of bacteria in the field of the microscope and the results obtained by the plate counts. Results obtained by this method in the hands of others do not appear to be satisfactory. The reasons for this were the personal equation of individual workers, speed and length of centrifugation, etc.

In 1911, Breed[6] introduced a direct microscopic method which has been used quite extensively. In his method 0.01 c c of milk was spread over 1 sq. c c, stained and examined directly under the microscope. Breed claimed that the number of bacteria determined by this method was quite comparable to the number determined by plate counts, if clumps instead of individual bacteria were counted. This method has the disadvantage of not being accurate with pasteurized milk or milks having low bacterial counts. However, if a rapid examination of raw milk was desired, this method could be used to determine both the number and character of the bacteria present with a fair degree of accuracy.

The reduction of dyes by bacteria in milk has been observed for some time. Duclaux[7] was probably the first to work on the reduction of dyes with special reference to milk. Since then, there have been quite extensive studies made of this method by Jensen,[8] Fred,[9] Barthel,[10] and others. All of these workers arrived at practically the same conclusions; namely, that (1) in a general way the reduction time is inversely proportional to the bacterial content; (2) that nearly all bacteria reduce dyes, and (3) that methylene blue gives the best and most uniform results. In order to obtain consistent results, it is essential (1) that the dye substance have a known formula; (2) that the strength of the solution used be constant; (3) that the solution be dilute enough not to interfere with the development of the micro-organisms, and (4) that the change in the appearance of the milk during reduction be quite pronounced. By comparing the time of reduction with the number of bacteria obtained by the plate method, a classification was formulated which can be used to correlate the reduction test with the bacterial count. Fred[9] has given milks the following rating: If reduction occurred in from 7-30 hours, the milk contains 1,000-500,000 bacteria per c c; in 2-7 hours, from 500,000-2,000,000; in one-quarter to 2 hours, about 15,000,000, and less than one-fourth hour from 10,000,000-150,000000. While the test was only very roughly quantitative, it was a rapid way of determining whether a milk was high or low in bacterial content. Its chief advantage lies in the fact that the test is easily made and is quite rapid.

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[3] Centralbl. f. Bakteriol., II, 1900, 7, p. 834.
[4] Jour. Infect. Dis., 1905, Suppl. 1, p. 209.
[5] Centralbl. f. Bakteriol., II, 1906, 16, p. 537.
[6] Centralbl. f. Bakteriol., II, 1911, 30, p. 337.
[7] Le Lait, Paris, 1894, p. 5.
[8] Centralbl. f. Bakteriol., II, 1908, 18, p. 211.
[9] Centralbl. f. Bakteriol., II, 1912, 35, p. 391.
[10] Ztschr. f. Untersuch. d. Nährungs- u. Genussmittel, 1911, 21, p. 513.

Each of the foregoing methods has its advantages which make its use possible in some place, either in the creamery, routine laboratory, or in research work. But each also has some disadvantages which prevent it from being entirely satisfactory. Most of the rapid methods at the present time are either roughly quantitative tests or are not applicable to all kinds of milk. It would seem that a method to be generally useful should be accurate, economical of time and money, and equally applicable to all milks. Frost,[11] in 1915, devised a method of determining the number of bacteria in milk which promises to be used quite extensively in the future. This method will be referred to as the "little plate" method in this text. Several advantages have been claimed for this method. Among these are: (1) rapidity, requiring only 4-8 hours to complete the analysis; (2) reasonable accuracy; (3) small amount of glassware and medium; (4) a permanent record, and (5) a differentiation of various types of bacteria present.

Up to the present time no quantitative work has been published on this method except by Frost. He has made comparative analyses on a considerable number of milks of varying bacterial content. The results appeared to correspond reasonably close with those obtained by the standard plate method and were obtained in about one-eighth the time. In addition to the quantitative count obtained, important information could be gained by studying the colony formation.

METHODS USED

In this study a series of milks was examined by making parallel counts by five methods of analysis, namely, direct microscopic; standard plate; lactose agar plate; little plate, and reduction test. The bacterial count obtained by the standard plate method, as recommended by the Committee on Milk Analysis appointed by the American Public Health Association, was used as a basis for comparison. Several dilutions were always made in order to have plates with the proper number of colonies. The plates were counted with a lens which magnified 3½ diameters. Plates were also made on a medium containing 1% lactose.

The direct microscopic plate was made by spreading 0.01 c c of milk evenly over an area of 1 sq. cm. on a clean glass slide. This milk was measured with an accurately calibrated pipet. The preparation was dried and, after the fat had been removed with xylol, it was fixed and stained. The number of groups or clumps of bacteria was counted, using the oil-immersion lens. The size of the microscopic field, for the combination of lenses used, was compared with the area of the smear. This gave a factor of 526,000. The average number of clumps in a field multiplied by this factor gave the bacterial content per c c.

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[11] Science, N. S., 1915, 42, p. 255. Jour. Infect. Dis., 1916, 19, p. 273.

In the reduction test the following formula was used:

Methylene blue—med. pur.................	1 gm.
Sodium chlorid............................	8.5 gm.
Distilled water............................	1,000 c c

One c c of this solution was put in a sterile test tube with 10 c c of the milk to be analyzed. The two were thoroughly mixed and then placed in the incubator at 37° C. and the length of time required to decolorize the methylene blue was noted. Numerous investigators have recommended that the methylene blue solution should be sterile, but tests were made using sterile and nonsterile methylene blue, and no appreciable difference in the reduction time could be noticed. The methylene blue was always measured, however, with a sterile pipet. No oil was placed on the surface of the milk in the test tubes as the cream came to the surface in a few minutes, thus partially excluding the air and preventing oxidation of the dye. The oil was necessary only when the tubes were observed at infrequent intervals. Frequent observations were made after the tubes were placed in the incubator.

In the little plate method, 1 c c of the sample of milk to be analyzed was mixed thoroughly with 1 c c of nutrient agar which had previously been liquefied and cooled to 45 C. In cases in which the milk was known to have a high bacterial content, the milk was diluted 10 times with sterile water before mixing with the liquefied agar. One-tenth of a c c of the mixture was then spread evenly over an area of 4 sq. cm. which had previously been marked off on a clean glass slide. The slide was flamed immediately before use to prevent contamination. When the agar had solidified, the slide was placed in a moist chamber and incubated at 37° C. The period of incubation was from 5-6 hours with good milk. At the end of the incubation period, the slides were removed and dried quite rapidly at nearly 100° C. on a hot plate. While the slides were still hot, they were immersed in a solution of 10% acetic acid in 95% alcohol for at least 1 minute. Without washing, the slides were immersed for 3-5 minutes in a staining solution, made by diluting 1 part of Loeffler's methylene blue with 4 parts of distilled water. This stained the colonies deep blue, while the background was light blue or not stained at all. The slides were then washed thoroughly and dried over a warm plate. The area of the microscopic field was determined for the low, high and oil immersion lenses. When the lower power was used, one colony per microscopic field represented 4,540 bacteria per c c; under high power, one colony per field represented 93,000 bacteria per c c, and under oil immersion, one colony per field represented 421,000 bacteria per c c. If the milk was diluted 1:10 before mixing with the agar, the above figures were multiplied by 10. The counting was done with the lens which gave the easiest field to count and in most cases this was the low power. With some of the good milks the entire plate was examined in order to get an accurate count.

SOURCES OF MILKS

The milks used were obtained from several sources so that the bacterial contents varied widely. This was desirable in order to determine if the rapid methods were applicable to all grades of milk. High grade milks were obtained at the university dairy barn (D. B.). Many samples were obtained from the university creamery (U. C.). The milk received at this place was representative of milk produced on dairy farms where no special care was used to produce a product of high quality. A few samples were obtained from dealers.

The pasteurized milks were obtained from local milk distributors (K. M. and Z.). A number of milks were also pasteurized in the laboratory (L.). This was done in small sterile bottles holding about 150 c c. A thermometer was inserted into the bottle so that the bulb was in the center of the milk. The milk was held at 62.5° C. for 30 minutes after the thermometer in the milk showed this temperature. In some cases, the samples were analyzed immediately, while others were placed in the icebox until the following day.

EXPERIMENTAL DATA

The milks analyzed have been divided into 4 classes according to the number of bacteria they contained as determined by the standard plate method. Class A consists of those containing less than 10,000 bacteria per c c; Class B, those containing between 10,000 and 100,000 per c c; Class C, those containing between 100,000 and 1,000,000 per c c, and Class D, those containing in excess of 1,000,000 per c c.

TABLE 1

CLASS A MILKS

Serial No.	Kind of Milk	Source	Number Bacteria per C C on				Reduction Time in Hours
			Breed Plate	Standard Plate	Lactose Plate	Little Plate	
1	Past.	L.	500,000	50	260	60	30
2	Past.	L.	500,000	100	170	120	30
3	Past.	L.	850,000	100	200	275	30
4	Past.	L.	500,000	200	100	80	30
5	Past.	L.	500,000	400	800	275	30
6	Past.	L.	500,000	800	1,700	225	80
7	Past.	L.	500,000	1,000	1,000	140	30
8	Raw	U. C.	500,000	1,000	2,000	1,200	24
9	Past.	L.	500,000	1,300	1,400	700	30
10	Past.	L.	Innumerable	1,600	2,700	4,900	18
11	Raw	U. C.	500,000	1,700	.....	1,500	30
12	Past.	L.	500,000	2,000	2,500	1,600	24
13	Raw	D. B.	500,000	2,100	.....	1,900	17
14	Raw	D. B.	500,000	2,500	4,600	3,500	3
15	Past.	L.	500,000	2,700	11,000	9,100	30
16	Past.	L.	1,100,000	3,400	5,600	4,500	30
17	Past.	L.	831,500	3,400	2,500	2,200	30
18	Raw	D. B.	500,000	5,000	.....	3,000	27
19	Raw	U. C.	500,000	5,000	5,000	6,400	15
20	Past.	L.	44,000,000	5,100	4,900	2,200	80
21	Past.	M.	500,000	6,000	7,000	5,500	22
22	Past.	L.	2,000,000	6,000	3,700	4,500	30
23	Raw	U. C.	500,000	8,800	4,600	5,400	10

From the foregoing table it can be seen that comparatively few counts were obtained by the direct microscopic method in this group of milks. Any figures given for milks with counts less than 600,000, or one bacterium per microscopic field, are only rough approximations.

The other figures given are for pasteurized milks and include dead bacteria and therefore show no relation to the results obtained by the cultural methods.

In the lactose agar plates, the counts obtained were higher 12 times, lower 6 times and twice alike. Averaging the counts obtained by the 2 methods, it will be found that the increase due to the lactose was approximately 20%.

TABLE 2

CLASS B MILKS

Serial No.	Kind of Milk	Source	Number Bacteria per C C on				Reduction Time in Hours
			Breed Plate	Standard Plate	Lactose Plate	Little Plate	
24	Past.	L.	38,900,000	13,000	18,900	29,000	30
25	Past.	M.	5,500,000	13,000	6,500	9,500	30
26	Raw	U. C.	500,000	15,000	30,000	31,400	13
27	Past.	L.	3,500,000	17,000	22,300	13,500	16
28	Past.	L.	Innumerable	17,000	10,200	18,200	30
29	Raw	D. B.	500,000	18,000	48,000	20,400	29
30	Past.	L.	69,000,000	18,300	65,000	54,000	30
31	Raw	U. C.	500,000	19,000	17,000	23,600	16
32	Raw	U. C.	500,000	22,000	26,000	24,600	14
33	Raw	U. C.	500,000	22,000	32,000	13,600	15
34	Raw	U. C.	16,000,000	23,000	35,000	22,700	12
35	Raw	U. C.	500,000	24,000	27,000	30,100	12
36	Raw	Z.	3,000,000	25,000	27,000	22,700	14
37	Raw	U. C.	500,000	25,000	27,000	27,700	12
38	Raw	U. C.	500,000	25,000	42,000	56,000	10
39	Raw	Z.	7,360,000	30,000	57,000	27,200	15
40	Raw	U. C.	500,000	30,000	......	68,000	18
41	Past.	L.	Innumerable	30,800	50,000	132,000	20
42	Past.	M.	Innumerable	31,000	86,000	200,000	18
43	Past.	M.	2,800,000	35,000	35,000	23,600	24
44	Raw	U. C.	500,000	35,000	21,000	22,600	15
45	Raw	U. C.	500,000	37,000	47,000	50,000	15
46	Raw	U. C.	500,000	37,000	40,000	28,000	12
47	Past.	L.	Innumerable	41,000	41,000	45,500	30
48	Raw	U. C.	500,000	41,000	133,000	91,000	10
49	Raw	U. C.	500,000	41,000	86,000	127,000	12
50	Raw	U. C.	500,000	44,000	......	63,600	15
51	Raw	K.	6,300,000	45,000	54,000	91,000	15
52	Past.	Z.	3,200,000	46,000	40,000	42,600	16
53	Raw	U. C.	500,000	46,000	99,000	132,000	12
54	Past.	Z.	11,000,000	52,000	12,000	29,000	24
55	Raw	U. C.	500,000	53,000	31,000	53,000	15
56	Past.	Z.	7,900,000	55,000	98,000	63,000	20
57	Past.	K.	24,000,000	57,000	90,000	54,000	22
58	Raw	D. B.	500,000	64,000	......	28,600	22
59	Raw	U. C.	500,000	64,000	75,000	55,800	4
60	Raw	U. C.	500,000	66,000	107,000	59,900	8
61	Raw	U. C.	500,000	69,000	91,000	77,100	12
62	Past.	Z.	3,200,000	72,000	34,000	54,500	20
63	Past.	L.	3,200,000	77,000	55,000	59,000	30
64	Past.	K.	1,300,000	79,000	11,000	68,100	30
65	Raw	D. B.	500,000	80,000	......	28,100	22
66	Raw	D. B.	500,000	80,000	......	26,800	24
67	Raw	U. C.	500,000	80,000	89,000	254,500	9
68	Raw	U. C.	500,000	87,000	67,000	115,300	10
69	Raw	U. C.	500,000	95,000	115,000	493,000	15
70	Past.	Z.	3,700,000	97,000	79,000	100,000	18

A long period of time was required to decolorize the methylene blue. If we disregard one specimen (Sample 14), which is evidently not comparable, the time varied from 10-30 hours, 17 out of the 23 samples required 24 hours or more. This test is apparently much more reliable than the Breed method for this class of milks, and the results would compare favorably with the standard plate figures. The little plate method gave results which are closely comparable to the standard plate count. Nine of the counts were higher on the little plates, while 14 were lower.

As in the preceding table, there were too few bacteria on the slide to be counted accurately by the direct microscopic method. The other figures given were all of pasteurized milks and have no direct relation to the living organisms.

The lactose agar plates again gave an increased count. The plates were higher 28 times and lower than the standard plates 12 times. The average increment due to the lactose was about 16%.

Thirty-nine of the 47 samples were put in the same class by the little plate and standard plate methods. Of the 8 put in different classes, 2 (Samples 68 and 70) were very close to the dividing line, hence were closely comparable. Twenty-six of the counts were higher on the little plates than on the ordinary plate method, while 20 of them were lower. One specimen (Sample 55) gave a similar count by the 2 methods.

The time required for the reduction of the methylene blue, excluding one sample (Sample 59), varied from 8-30 hours. Forty-one of the 47 samples in this class required 12 hours or more.

Nine of the samples counted by the direct microscopic method were raw milks and the figures obtained for these compare favorably with the standard plate counts in 5 cases. As in the previous tables, none of the 14 pasteurized milks counted gave comparable counts.

The lactose plates again gave larger numbers than the plain agar, the increased count being slightly over 20%. It is worthy of notice, however, that this average increase was due to the marked increase of a few samples for 22 of the milks gave a lower count than the standard plates and only 12 higher.

Of the 38 samples in this class, 31 of them were put in the same class by the little plate and ordinary plate method. Of the 6 put in different classes, all were quite closely comparable and were very much closer than duplicates on ordinary plates.

The average methylene blue reduction time was much shorter than in the preceding classes. The time varied from 8-30 hours, while 22 of the 38 samples required between 7 and 12 hours.

TABLE 3

CLASS C MILKS

Serial No.	Kind of Milk	Source	Number Bacteria per C C on				Reduction Time in Hours
			Breed Plate	Standard Plate	Lactose Plate	Little Plate	
71	Raw	U. C.	9,500,000	100,000	109,000	63,000	12
72	Past.	L.	4,600,000	101,000	94,000	218,000	16
73	Raw	U. C.	500,000	102,000	122,000	363,000	8
74	Raw	U. C.	500,000	103,000	384,000	213,000	10
75	Past.	K.	6,300,000	110,000	51,000	183,000	15
76	Raw	U. C.	500,000	118,000	117,000	114,000	9
77	Raw	U. C.	500,000	125,000	115,000	194,000	10
78	Past.	L.	Innumerable	126,000	121,000	63,200	20
79	Raw	D. B.	500,000	127,000	....	180,000	15
80	Past.	K.	4,300,000	130,000	127,000	132,000	16
81	Raw	U. C.	500,000	132,000	251,000	76,000	12
82	Raw	U. C.	500,000	136,000	107,000	180,000	12
83	Raw	U. C.	500,000	150,000	250,000	228,000	8
84	Past.	Z.	8,400,000	160,000	210,000	100,000	18
85	Past.	M.	6,700,000	167,000	250,000	155,000	20
86	Past.	L.	42,000,000	175,000	150,000	240,000	22
87	Past.	K.	6,800,000	200,000	174,000	651,000	16
88	Past.	M.	2,900,000	210,000	100,000	145,000	16
89	Raw	U. C.	500,000	215,000	....	96,000	12
90	Past.	M.	6,300,000	220,000	116,000	153,000	24
91	Raw	U. C.	500,000	232,000	174,000	400,000	8
92	Past.	M.	5,800,000	234,000	216,000	218,000	16
93	Raw	U. C.	2,100,000	250,000	800,000	960,000	8
94	Past.	K.	4,500,000	287,000	197,000	368,000	30
95	Raw	U. C.	500,000	290,000	267,000	324,000	10
96	Raw	U. C.	500,000	290,000	420,000	353,000	12
97	Raw	U. C.	500,000	327,000	395,000	359,000	10
98	Raw	U. C.	1,400,000	352,000	288,000	530,000	8
99	Past.	L.	15,800,000	390,000	680,000	744,000	16
100	Past.	K.	31,600,000	420,000	400,000	1,020,000	12
101	Raw	U. C.	5,900,000	470,000	390,000	459,000	7
102	Raw	U. C.	1,100,000	570,000	....	922,000	12
103	Raw	U. C.	1,600,000	613,000	555,000	1,000,000	10
104	Raw	U. C.	500,000	620,000	420,000	1,520,000	12
105	Past.	K.	4,200,000	650,000	520,000	482,000	18
106	Raw	U. C.	1,000,000	680,000	....	817.000	12
107	Past.	L.	32,000,000	680,000	2,100,000	2,410,000	26
108	Raw	U. C.	1,470,000	820,000	750,000	540,000	8

Twenty-five counts were made on the direct microscopic plate, and of these, 20 were quite closely comparable with the standard plates.

Less than half of these milks were plated on lactose agar, but the figures obtained were almost exactly double those on the standard plate method and 6 of the 8 samples gave higher counts. Of the 28 samples in Class D, only 4 were put in different classes by the standard plate and little plate method.

The reduction time was short in this group of milks varying from one-half hour to 10 hours. Eighteen of the 28 samples required 4 hours or less.

From the summary given in Table 5 it can be seen that the milks were placed in the same classes in nearly every case by the standard, lactose agar and little plate methods. Several of those which have been put in separate classes were very close, as they were very near the division line between the two classes. No milks were put in Classes A or B with the direct microscopic method, as these classes contain so few bacteria per c c that an accurate count was impossible. A large number of the samples were put in Class D. These samples constituted the pasteurized milks and those whose bacterial content was very high. A considerable number of those put in Class D gave very close counts with the standard plates. A few of the Breed plates were seeded with clumps and single bacteria so that counts were impossible.

The data are shown also in Charts 1 and 2. The bacterial contents are represented by the logarithms of the numbers rather than by the actual numbers, which permits the plotting of all the different grades of milk in a single curve.

TABLE 4

Class D Milks

Serial No.	Kind of Milk	Source	Number Bacteria per C C on				Reduction Time in Hours
			Breed Plate	Standard Plate	Lactose Plate	Little Plate	
109	Raw	U. C.	2,700,000	1,000,000	1,130,000	2,300,000	6
110	Raw	U. C.	4,700,000	1,400,000	1,600,000	5,900,000	4
111	Raw	U. C.	500,000	1,500,000	2,300,000	1,900,000	10
112	Raw	U. C.	2,100,000	1,700,000	....	1,670,000	4
113	Raw	U. C.	2,630,000	1,800,000	....	1,960,000	5
114	Raw	U. C.	2,100,000	1,840,000	....	1,500,000	8
115	Raw	U. C.	680,000	2,000,000	....	640,000	10
116	Raw	U. C.	12,600,000	5,300,000	....	6,300,000	3
117	Raw	U. C.	15,700,000	5,400,000	....	3,400,000	4
118	Raw	U. C.	5,400,000	5,600,000	....	2,500,000	4
119	Raw	U. C.	100,000,000	6,500,000	28,900,000	4,400,000	2
120	Raw	U. C.	100,000,000	6,600,000	12,800,000	3,200,000	3
121	Raw	U. C.	8,900,000	7,100,000	....	272,000	3
122	Raw	U. C.	13,500,000	8,400,000	3,000,000	6,000,000	6
123	Raw	U. C.	11,000,000	12,900,000	....	590,000	3
124	Raw	U. C.	13,700,000	12,900,000	....	376,000	2
125	Raw	U. C.	21,000,000	12,900,000	....	5,700,000	3
126	Raw	U. C.	11,000,000	13,100,000	....	4,130,000	8
127	Raw	U. C.	16,300,000	13,500,000	....	14,400,000	6
128	Raw	U. C.	10,000,000	13,900,000	....	2,860,000	2
129	Raw	U. C.	18,900,000	13,900,000	....	7,600,000	3
130	Raw	U. C.	14,200,000	14,600,000	....	14,880,000	5
131	Raw	U. C.	9,500,000	15,600,000	....	9,300,000	9
132	Raw	U. C.	61,500,000	16,700,000	60,000,000	48,000,000	2
133	Raw	U. C.	13,000,000	17,000,000	....	5,400,000	2
134	Raw	U. C.	12,300,000	17,200,000	9,100,000	13,700,000	2
135	Raw	U. C.	Innumerable	148,000,000	....	143,000,000	½
136	Raw	U. C.	Innumerable	160,000,000	....	155,000,000	⅓

TABLE 5

Distribution of Milks into the Four Classes by the Various Methods Used

	Direct Microscopic Plates	Standard Plates	Lactose Plates	Little Plates
Class A..............................	0	23	20	24
Class B..............................	0	47	41	43
Class C..............................	3	38	34	42
Class D..............................	67	28	9	27
Total..........................	70	136	104	136

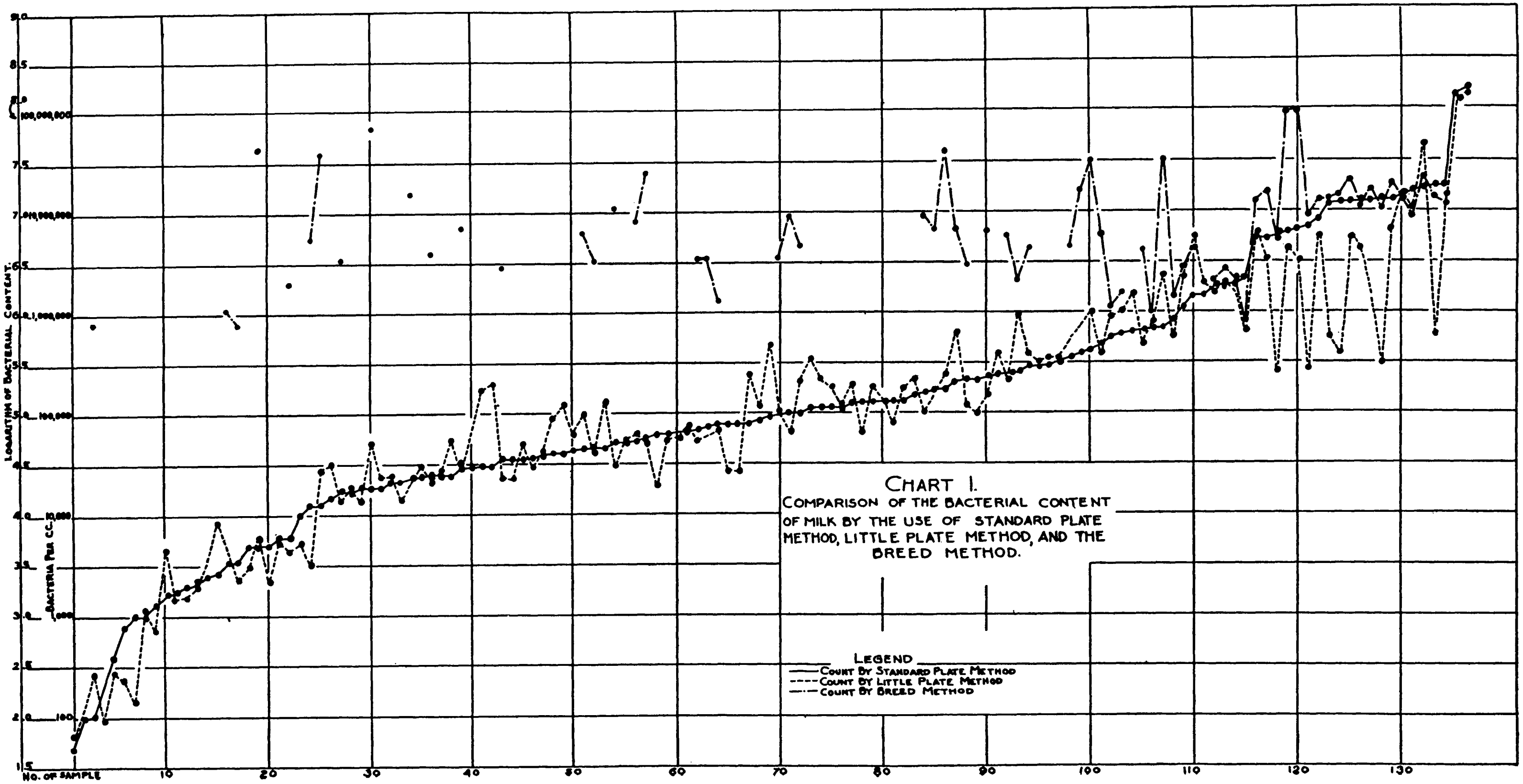

CHART I.
COMPARISON OF THE BACTERIAL CONTENT OF MILK BY THE USE OF STANDARD PLATE METHOD, LITTLE PLATE METHOD, AND THE BREED METHOD.

LEGEND
—— COUNT BY STANDARD PLATE METHOD
- - - - COUNT BY LITTLE PLATE METHOD
—·—· COUNT BY BREED METHOD

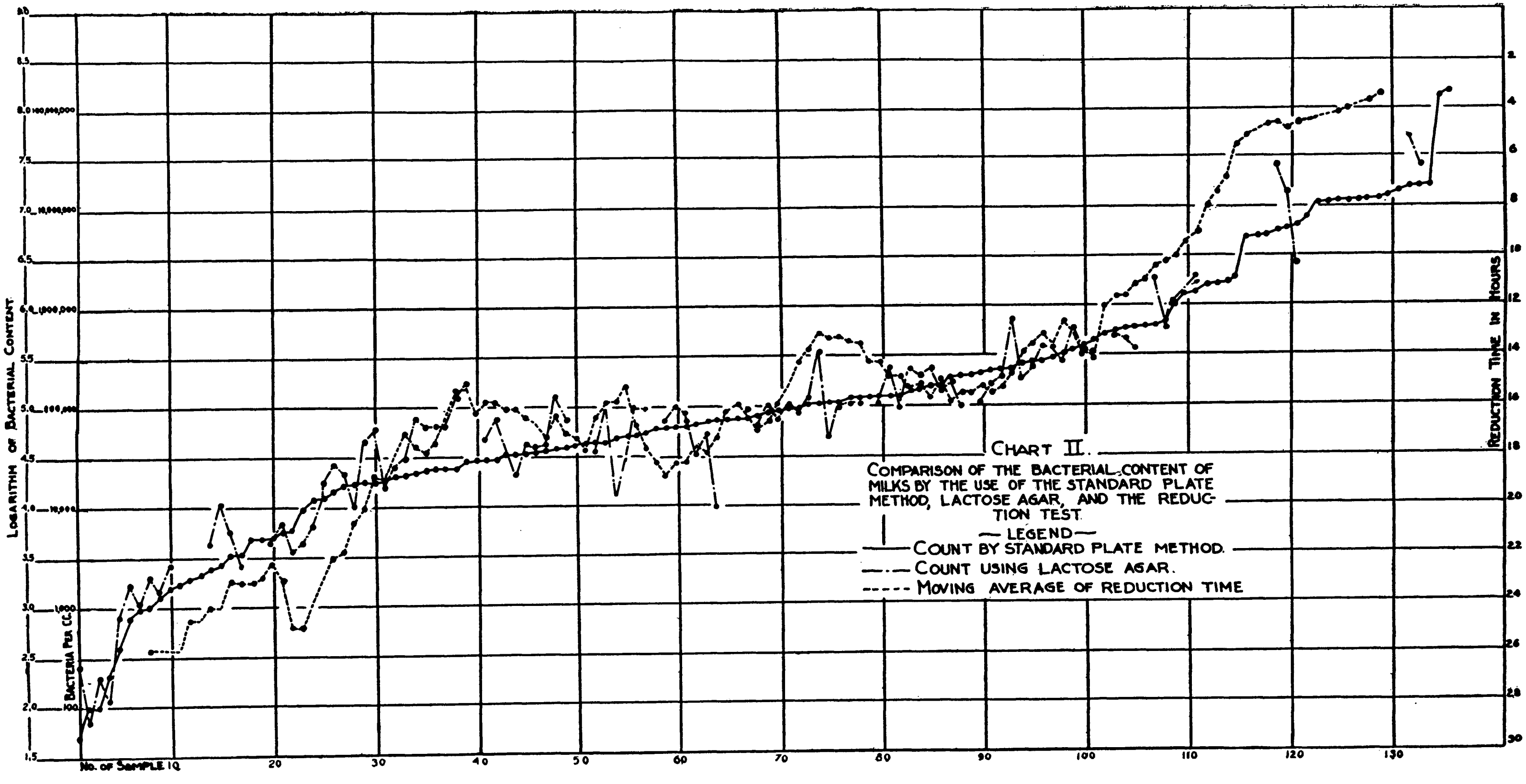

CHART II.

COMPARISON OF THE BACTERIAL CONTENT OF MILKS BY THE USE OF THE STANDARD PLATE METHOD, LACTOSE AGAR, AND THE REDUCTION TEST.

— LEGEND —

——— COUNT BY STANDARD PLATE METHOD.

—·— COUNT USING LACTOSE AGAR.

----- MOVING AVERAGE OF REDUCTION TIME

The milks were arranged in an ascending series by the counts obtained on the standard plates and the results obtained by the other methods were indicated as variations from this.

In Chart 1 the direct microscopic count and the little plate count were compared with the standard plates. The curve representing the little plate followed the curve for the standard plate determinations throughout the course, while the direct microscopic curve was in nowise comparable until the germ content exceeded a million per c c.

In Chart 2 a comparison was made of the lactose agar and the reduction test with standard plate method. The figures for the lactose and plain agar were represented by the logarithms as in Chart 1, but it seemed best to represent the reduction time by a "moving average."

The results of the reduction time in the tables appear to be very irregular, but when a large number of results were observed, there seemed to be quite a close correspondence between the reduction time and the standard plate counts. Since the variations in the reduction time appeared to be very irregular, a better idea of the comparison of this method with standard plates was obtained if the "moving average" of the reduction time was plotted, rather than the actual reduction time. King[12] says: "One of the best methods of smoothing certain varieties of histograms is to use a moving average to obtain a trend." The first step in the computation of the moving average was to obtain the average reduction time of the first 15 samples. This constituted the moving average for the eighth sample. By adding the sixteenth sample and dropping the first sample, the average can be found **for** the ninth sample. By successively dropping the first sample and adding one each time, the average could be determined to the end of the series. This method does away with single large fluctuations and made the two methods more comparable.

DISCUSSION

Breed Method.—The direct counts were not comparable with the counts by the other methods except with milks having quite a high bacterial content. When the bacteria were less than 500,000, the accuracy of the direct count was doubtful. However, the direct count served as a rapid means of determining whether a raw milk was high or low in bacteria. The application of the direct count to pasteurized milks does not seem feasible until a method has been devised to differentiate between dead and living bacteria.

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[12] Elements of Statistical Method, 1912.

The results obtained by the direct count are much higher than the standard plate counts on milks containing few bacteria. Several reasons for this difference are possible. Not all of the organisms in milk grow on plain agar. There may be some dead organisms in the milk which were counted under the microscope, but will not show on the standard plate. This was especially true in pasteurized milks.

While the correspondence between the direct count and the plate method was not close, the direct count served as an indication of the total number of bacteria regardless of whether they were active or not. It gave in a short time a general idea of the number of bacteria in the milk.

Lactose Agar.—While a good many of the counts on the lactose agar were lower than the corresponding count on plain agar, these variations were mostly within the range of experimental error. The same was not true of those having a count higher than on the plain agar, since many of the variations were 200 and 300% above those on plain agar.

It would thus seem that the lactose agar furnished a more suitable medium, especially for some milks. It also appeared that the colonies were considerably larger on lactose agar than they were on plain agar, so much so that a lens was not required in counting the lactose agar plates. This confirmed the findings of Sherman.[13]

Little Plate. — It can be readily seen that the correspondence between the little plates and the standard plates was quite close. The average count on the little plate was about 25% higher than on the standard plate. This higher count might be explained on the supposition that some bacteria in milk grew on the little plates but not on the standard plates. This was reasonable, since the little plates have a high percentage of milk in the culture medium. Milks having a high bacterial content appeared to give a lower count on the little plates than on the standard plates. This was possibly due to the fact that the clumps of bacteria were broken up when high dilutions were used in standard plates. This possibility of breaking up the clumps was very much less in the little plates, since only one dilution was necessary.

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[13] Jour. Bacteriol., 1916, 1, p. 481.

A careful study of the tables and charts also showed that there was a somewhat closer correspondence between the little plates and the lactose agar counts than there was between the little plates and the plain agar counts.

Reduction Test.—The reduction test, when used to measure the bacterial content of milk, showed considerable variation. However, it was a rapid and very easy method for determining the activity of the bacteria in the milk. While the time of reduction in this investigation appeared somewhat longer than that obtained by other workers, a correspondence does exist between the time of reduction and the number of bacteria. Additional tests not recorded here appear to show that the strength of the methylene blue solution can be reduced, thus making the time of reduction shorter. This is a matter of considerable practical value.

This test, in contrast to all other methods used to determine the bacterial content of milk, was very economical of equipment and very simple in technic.

SUMMARY

One hundred and thirty-six milks, varying in bacterial content from 50-160,000,000 per c c, have been analyzed by 5 different methods: Direct microscopic, standard plate, lactose plate, little plate (Frost), and reduction test.

In milks containing less than 1,000,000 bacteria per c c the 3 methods — standard plate, lactose plate and little plate — gave closely comparable results. The lactose plates on the whole gave results slightly higher than the plain agar plates, and these results seemed to follow the trend of the little plate counts somewhat more closely than the standard plates.

The direct microscopic count was less reliable with milks having a low bacterial content than it was with poor milks.

In milks containing upward of 1,000,000 bacteria per c c, the lactose plates were found to be considerably higher (50%) than the standard plates, the little plates tended to run somewhat lower, while the direct microscopic count gave highly satisfactory results.

The time of the reduction test varied from one-half hour to 30 hours. Occasionally inexplicable variations occurred in the different classes of milks, but when these were smoothed out by applying a "moving average," a curve was obtained which closely approximates those obtained by the other methods.

CONCLUSIONS

When the bacterial content of a milk was low (less than a million) all of the methods used gave satisfactory results except the direct microscopic count.

The little plates furnished results in one-eighth to one-fifth of the time required by the other culture methods.

The bacterial content of highly contaminated milks could be quickly and satisfactorily determined by either the direct microscopic method, except in case of pasteurized milk, or the reduction test.

The character of the bacterial flora was revealed in a striking way by both the little plate and the direct microscopic count.